Protein Metabolism in Thyroid Glands of Euthyroid, Thiouracil-fed and Hypophysectomized Rats after Cortisone Treatment[1] (34781)

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Previous studies in our laboratory showed that cortisone treatment of intact rats markedly decreases thyroid gland weight, the pool of (free) amino acids, and the ability to incorporate ^{3}H-leucine into protein (1, 2). In contrast, administration of thiouracil significantly increases all of the above parameters. When cortisone administration was superimposed on goitrogen-feeding, a further significant increase in both gland weight and ability to incorporate labeled amino acids was observed, but the pool of (free) amino acids was unaltered (2, 3). However, the previous studies do not suggest an explanation of the different responses to cortisone. Therefore, in the present studies we have attempted to explain the action of cortisone by utilizing hypophysectomized, pituitary-replaced animals in conjunction with *in vitro* experiments on the transport of the nonmetabolizable amino acid cycloleucine. Evidence is presented indicating that cortisone action in the nongoitrogen-fed rat is not mediated by the pituitary gland and, furthermore, that neither cortisone nor thiouracil alone or in the combination after pretreatment *in vivo*, affects the transport of cycloleucine into cells of the thyroid gland *in vitro*.

Methods. In vivo. Young male rats (125 g) (Badger Rat Corp., Madison, Wisconsin) were hypophysectomized and maintained on a diet of Lab-blox and water containing 5% Cerelose and 0.85% NaCl. Three days after hypophysectomy, pituitary replacement, consisting of a daily sc injection of 1 rat pituitary homogenized in 0.2 ml of 0.85% NaCl, was initiated in half the animals. Five days after commencement of pituitary therapy, the rats were divided into four groups. One group continued to receive only pituitary replacement therapy (H + P); the second received cortisone in addition (H + P + C); third, no therapy (H); and the fourth, only cortisone therapy (H + C) (Merck Sharp and Dohme Cortone Acetate, 5 mg/day sc).

Food and Cerelose were withdrawn 16 hr before killing on the eighth day of cortisone administration. Two hr before killing, rats were injected ip with 1 μCi/g of body weight of ^{3}H-*l*-leucine-4,5 (5.0 Ci/mmole, New England Nuclear). Rats were killed by exsanguination and the organs were frozen for analysis. Tissue analysis for DNA, protein, and estimation of ^{3}H-leucine incorporation was performed as previously reported (1) with the modification that each thyroid was analyzed individually. Animals were eliminated if at autopsy evidence of pituitary tissue was found. A duplicate experiment was performed using young male Rolfsmeyer rats.

In vitro. Young male rats (125 g) (Rolfsmeyer Rat Co., Madison, Wisconsin) were divided into 2 groups. One was maintained on Lab-blox and was considered "euthyroid" (Eu); the other one a mixture of ground Lab-blox containing thiouracil (0.5% by wt) (Tu). On the 10th day of thiouracil feeding, cortisone treatment of half the Eu animals (Eu + C) and half the Tu animals (Tu + C) was initiated.

On the 12th day of cortisone treatment,

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the rats were killed by exsanguination, the thyroids were removed and quickly placed in sterile 30-ml culture flasks containing 4.9 ml of Eagle's minimal essential medium 10 H (MEM). The flasks were gassed with 95% O_2–5% CO_2 and placed in a Dubnoff metabolic shaking incubator at 37.5°. After 0.5 hr, 0.1 ml of Eagle's medium containing either 10 μCi of ^{3}H-inulin (200 mCi/g) or 20 μCi ^{3}H-aminocyclopentane-1-carboxylic-2,5-acid (cycloleucine), 81.3 mCi/mmole was added to each flask. The flasks were then immediately re-gassed for 30–60 sec and again at 1- and 2-hr intervals during the incubation. After 3 hours, the thyroid lobes were removed from the flasks and rinsed twice with 0.85% NaCl. The lobes incubated in cycloleucine were weighed and frozen. Those incubated in inulin were placed on weighed pre-dried squares of filter paper. The glands and filter paper were weighed, placed in the vacuum dessicator at 65° and dried to constant weight. For analysis, each lobe was thawed and homogenized individually in 0.5 ml of H_2O. The homogenate was rinsed into a 12-ml tube to a volume of 1.0 ml with H_2O, covered with a marble, heated in a boiling water bath for 5 min, and then centrifuged for 10 min at 1000*g*. A 0.5-ml sample of the supernate was mixed with 10.0 ml of a scintillation mix (10% Naphthalene, 1% PPO, 0.025% dimethyl POPOP) and counted in a Nuclear Chicago model 725 liquid scintillation counter. After determination of dry weight, inulin incubated lobes were homogenized and the radioactivity was determined as above. An estimate of total distintegrations per sample was obtained by adding an internal standard and recounting the sample.

On the assumption that inulin in the media comes into equilibrium with the extracellular space of the incubated thyroid, the fraction of tissue occupied by extracellular fluid is estimated from the ratio of the distintegrations per minute of ^{3}H-inulin per milligram of tissue to that per microliter of media. The total count of cycloleucine in a gland was corrected for the fraction of cycloleucine in the interstitial spaces. The ratio of the counts of cycloleucine in the cell to the counts of cycloleucine in the media is used as an index of accumulation.

Results. In vivo. Body weight. Hypophysectomy results in cessation of growth. However, the group given pituitary replacement therapy gained weight in a manner similar to that of unoperated control animals (1), and their final body weights were 50% higher than those of the hypophysectomized only group. When cortisone was administered to pituitary-maintained animals, it prevented growth, whereas, when administered to H animals, it produced a net loss in body weight.

Organ weight. Final liver weight, like body weight, was depressed by hypophysectomy. Pituitary replacement therapy resulted in liver weights which were the same as those reported previously for intact rats (1). Administration of cortisone resulted in an increase in liver size in both H and H + P animals.

Thyroid weights of the hypophysectomized pituitary-replaced rats were equal to those observed in intact rats of similar age (Fig. 1) (3). As in the case of intact animals (1), corticoid administration depressed thyroid weight in pituitary replaced rats.

Amino acid incorporation. Pituitary replacement resulted in a "normal" incorporation of ^{3}H-leucine into purified protein of the

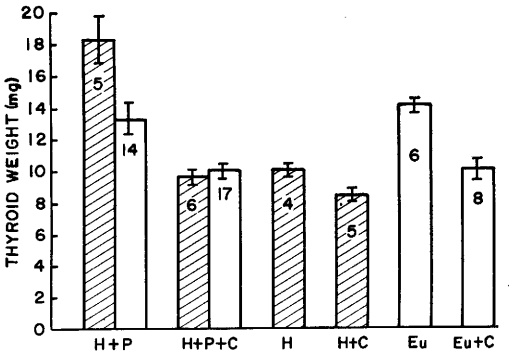

FIG. 1. Comparison of thyroid weights under different hormonal stimuli: (shaded bars), an experiment using Badger rats; (clear bars) a second experiment using Rolfsmeyer rats. Significant differences ($p < 0.01$) are found between H + P and H + P + C and between H + P and H. For the Rolfsmeyer rat experiment, no significant difference is found between the H + P and Eu animals.

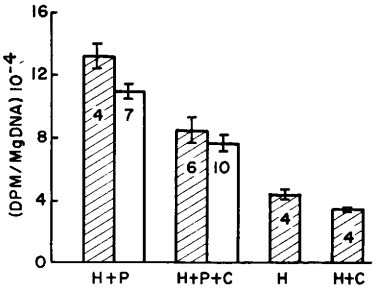

FIG. 2. Comparison of the incorporation of ^{3}H-leucine into thyroid protein under different hormonal stimuli: experiments are denoted as in the legend of Fig. 1. The difference between H + P + C and H + P and between H + P + C and H yield t values with a $p<0.01$. The t value for the difference between H + P and H has a $p<0.05$.

thyroid glands on a per milligram of DNA basis, whereas injection of cortisone for 8 days to the replaced rat reduced the ability of the thyroids to incorporate labeled leucine (Fig. 2). Pituitary replacement therapy thus appeared to maintain thyroid gland isotope incorporation at the "normal" level. Furthermore, in two separate experiments the thyroid glands of the hypophysectomized, pituitary-replaced rats responded to prolonged treatment with cortisone in a manner similar to those of intact animals.

In vitro. The time course of ^{3}H-inulin uptake by the thyroid gland tended toward a plateau between the second and fourth hours of incubation. The uptake of ^{3}H-cycloleucine reached a maximum at 30 min (the earliest time interval studied) and remained near this level through 4 hr. A 3-hr incubation time was used, therefore, in all subsequent studies with inulin or cycloleucine.

TABLE I. Effect of *in Vivo* Administration of Cortisone and Thiouracil on Thyroid Gland ^{3}H-Inulin Space[a] Determined *in Vitro*.

Treatment	No. of rats	Inulin space
Euthyroid	5	0.35 ± 0.035[b]
+ cortisone	6	0.35 ± 0.046
Thiouracil	5	0.22 ± 0.030
+ cortisone	6	0.29 ± 0.025

[a] Estimate of fractional extracellular space of the organ.

[b] Values ± standard deviation.

Inulin space. The inulin space of the thyroid glands of Tu-fed rats was markedly reduced compared to glands of Eu animals ($p<0.01$) (Table I). This finding agrees with the cellular hypertrophy that occurs in the thyroids of goitrogen-fed animals. Administration of cortisone partially prevented the marked reduction in the thyroid gland extracellular space associated with Tu-treatment. In fact it was significantly larger ($p<0.01$) than that of thyroids from rats fed Tu-only. However, the extracellular space of the thyroids of Tu + C animals was still less ($p<0.05$) than that of either Eu or Eu + C rats. The inulin space of Eu animals was not altered by cortisone treatment.

Cycloleucine accumulation index. Cycloleucine apparently crosses cell membranes in a manner similar to that of normal amino acids. In addition, it is virtually nonmetabolized (4). No difference was found in the ability of this nonmetabolizable amino acid to enter the intracellular pool of the thyroid glands of the variously treated rats (Table II).

TABLE II. Thyroid Gland Intracellular Accumulation Index[a] of ^{3}H-Cycloleucine *in Vitro* of Rats Treated with Cortisone and Thiouracil *in Vivo*.

Treatment	No. of rats	Index
Euthyroid	10	0.85 ± 0.23[b]
+ cortisone	8	0.72 ± 0.20
Thiouracil	11	0.80 ± 0.11
+ cortisone	10	0.79 ± 0.20

[a] The ratio of cycloleucine counts in the cell to the media counts.

[b] Values ± standard deviation.

Discussion. The present study was undertaken in an effort to explain how prolonged cortisone administration increases both thyroid gland weight and incorporation of labeled amino acids in Tu-fed rats and decreases both in the Eu-animal (1). Cycloleucine was used to investigate whether cortisone affects amino acid transport across thyroid cell membranes and whether this transport is modified by Tu-administration. The lack of effect of the various *in vivo* treatments on *in vitro* cycloleucine uptake tends

to support the conclusion that the differences in thyroid protein metabolism observed were not the result of altered amino acid transport.

It should be noted, that, on the one hand, TSH has been reported to stimulate transport of another nonmetabolizable amino acid—amino isobutryic acid into thyroid slices (5); while on the other, it has been observed not to produce a discernible effect on transport of amino acids, including cycloleucine (6).

In intact Tu-fed animals, a cortisone-induced increase in TSH release as a consequence of ACTH inhibition could account for our previous observation of enhanced protein synthesis in thyroid which would agree with the observations of others (7–9).

However, invoking a pituitary feedback to explain the mechanism of cortisone action is not consistent with the observation that the rate of thyroid amino acid incorporation is decreased in Eu + C animals. Nevertheless, in an attempt to test the feedback hypothesis, hypophysectomized rats receiving pituitary gland replacement were used. Their decreased thyroid size and the reduced ability of their thyroids to incorporate labeled leucine argue against major pituitary involvement. Still the possibility exists that since the pituitary homogenate contained TSH, the action of cortisone is mediated by interference with the action of TSH on target issue which in turn is modified by Tu-administration.

Although the current experiments do not demonstrate either a direct action of cortisone on the thyroid cell membrane or one mediated via the pituitary gland, it is possible that the thyroid responses in Eu + C rats (1) are the result of an inadequate supply and pattern of amino acids after prolonged corticoid administration. The observation that 10 days of cortisone treatment to Eu rats reduces the plasma (free) amino acid pool by 20% and the skeletal muscle pool by 40% (10) agrees with such a postulate. The apparent contradictions of Tu + C thyroids could be explained in two ways. Thiouracil might alter the priority of the thyroid gland in competing with other organs for available amino acids, or, in modifying the protein catabolic potential of cortisone on liver (3), it might enable greater quantities of amino acids released from the liver to reach the thyroid gland. Studies to determine the validity of the above hypotheses are currently in progress.

Summary. Chronic administration of cortisone (5 mg/day) to hypophysectomized pituitary replaced rats resulted in decreased: (a) body weight; (b) thyroid weight; (c) incorporation of ^{3}H-leucine into thyroid protein; and (d) an increase in liver weight similar to that seen in unoperated rats injected with adrenocorticoids. These data indicate that cortisone is equally effective in intact and hypophysectomized rats receiving pituitary replacement therapy but argue against a major pituitary involvement. Incubation of thyroid gland lobes *in vitro* with ^{3}H-inulin indicated that the extracellular gland space of thiouracil-fed rats was markedly reduced below that of either euthyroid or euthyroid plus cortisone animals ($p<0.01$). When thiouracil-fed rats were given cortisone their gland inulin space was not reduced as much, although it was still significantly smaller ($p<0.05$) than that of glands from rats of either euthyroid group. Cortisone treatment, however, had no effect on the inulin space in glands of euthyroid animals. The ability of ^{3}H-cycloleucine to enter the thyroid gland intracellular pool under *in vitro* incubation condition was unaffected by any of the prior *in vivo* treatments. It is suggested that chronic cortisone treatment may affect thyroid gland protein biosynthesis indirectly by altering its available supply and pattern of amino acids.

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